Scale-up of Thermophilic Ionic Liquid-tolerant Cellulase Cocktail for Lignocellulosic Biofuel Production

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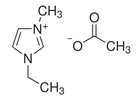
Abstract

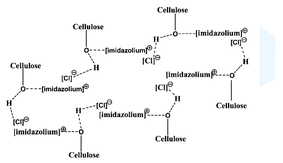
Ionic liquid pretreatment of lignocellulosic biomass shows great potential in effectively reducing the biomass recalcitrance with less enzyme requirement for saccharification of biomass into sugars. However, commercially available cellulase enzymes are sensitive to residual ionic liquids from pretreatment necessitating extensive and expensive washing steps to remove ionic liquids from pretreated material. As a result, high costs associated with the extensive pretreatment process followed by washing and separate hydrolysis has become one of the main barriers for commercialization. Consolidation of pretreatment and enzymatic saccharification would address the current techno-economic challenges related to high cost of separate pretreatment and saccharification. To accomplish this, researchers at Joint Bioenergy Institute (JBEI) have developed a thermophilic ionic liquid-tolerant cellulase cocktail called “JTherm” for biofuel production. The cocktail can tolerate up to 20-30% ionic liquid over a range of temperatures, 50-70 deg C, when incubated at pH 5.5 for 72 hours.

In this study, we present the results from the scale-up of production of JTherm cocktail in 75L bioreactors at Advanced Biofuel Process Demonstration Unit (ABPDU). Initial results showed excellent scalability of the process with enzyme yields similar to that of shake flasks and further optimization would expand the cellulolytic enzymes landscape to integrate various ionic liquid pretreatment technologies overcoming barriers to production of economically viable lignocellulosic biofuels.

Pretreatment : Ionic Liquids as Catalysts



1-ethyl-3-methyl imidazolium acetate



Hydrogen Bonding between ILs and Cellulose (H. Tadesse et.al, 2011)

Advantages:

- Negligible vapor pressure
- High solvation capacity
- Reduces biomass recalcitrance
- Enhances enzymatic hydrolysis
- No poisonous gases during pretreatment

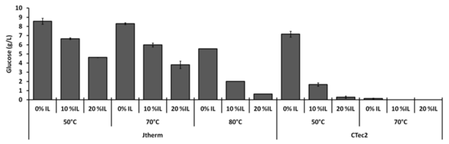
Disadvantages:

- Residual ILs inhibit sacharification and fermentation
- Expensive ionic liquids require efficient recycling
- Requires extensive washing leading to high costs
- High recycling cost due to extensive washing

JTherm Cocktail Vs. Ctec2

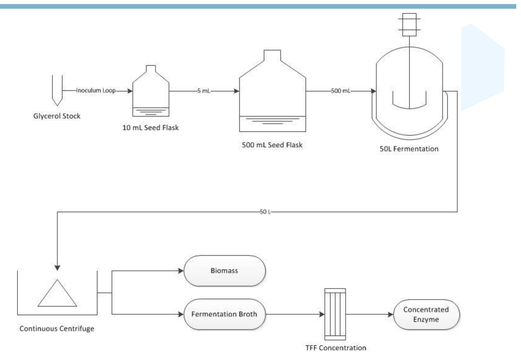
JTherm Enzyme Cocktail consists of

- Secretome from thermophilic community culture
- Cellobiohydrolase
- Beta-glucosidase

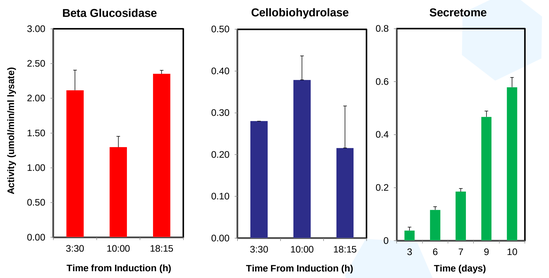


Activity comparison between JTherm and Ctec2 cocktails in the presence of ionic liquid at pH 5.5 for 72 hours with shaking (Joshua I. Park. et.al, in review)

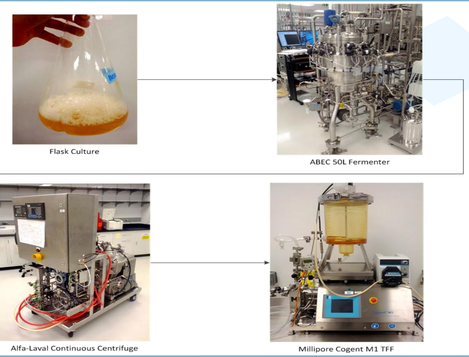
Production of JTherm Cocktail : Process Flow Diagram



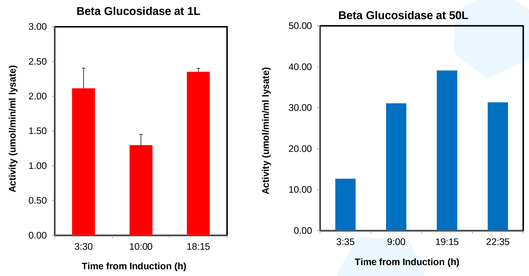
Enzyme Production in Bench Top Reactors



Scale-up of JTherm Cocktail Production



Scale-up of Beta-glucosidase Production in 50L



Greater than 10 fold increase in enzyme yields at 50L scale

Conclusions and Future Work

Conclusions:

- **JTherm cocktail shows higher ionic liquid-tolerance and thermostability than Ctec2**
- **Confirmed the activity of all the enzymes in the cocktail at 1L scale**
- **Successfully scaled up the beta-glucosidase production to 50L scale with 10 fold increase in enzyme yields.**

Future Work

- **Reconfirm the increased enzyme yields at 50L**
- **Scale-up of cellobiohydrolase and secretome production**
- **Expression and secretion of the all the enzymes of the cocktail in a single host microorganism**
- **Optimization of enzyme expression and yields**
- **Scale up of the enzyme cocktail production in 300L reactor**

Pretreatment Equipment at ABPDU

Parr Reactors

Hydrolysis Reactors

Basket Centrifuge

50L Hydrolysis Reactors

Fermentation Equipment at ABPDU

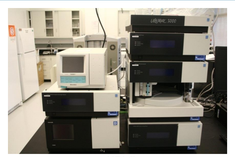
4L Bioreactors

20L and 50L Bioreactors

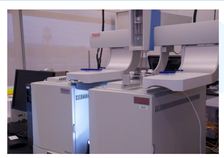
300L Bioreactors

Continuous Centrifuge

Analytical Equipment at ABPDU

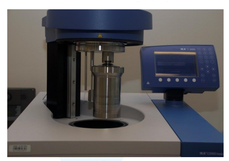


HPLC



Gas Chromatography

Akta Avant Protein Purification



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Acknowledgements

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- John Gladden
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